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CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

007374

DEC 2 1988

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Tebuthiuron oncogenicity studies
HED Project No. 9-0927 Caswell No. 366 AA

FROM: Quang Q. Bui, Head
Review Section I
Tox Branch-HFAS/HED (TS-769C) 11/30/88

TO: Robert Taylor, PM # 25
Registration Division (TS-767C)

THRU: Marcia Van Gemert, Acting Chief
Tox Branch - HFAS
Health Effects Division (TS-769C) 12/1/88

THRU: William L. Burnam, Acting Director
Health Effects Division
Office of Pesticide Programs (TS-769C)

Registrant: Elanco
Greenfield, Indiana

BACKGROUND INFORMATION:

Two chronic/oncogenicity studies with Tebuthiuron were conducted in rodents and were previously submitted to the Agency for review. Both studies (1) Rat chronic feeding study No. R-603 and R-613 [MRID 00020714] and (2) Mouse chronic feeding study No. M-9153 and M-9163 [MRID 00020717] were classified as Core Supplementary Data in light of a number of deficiencies as cited in the Toxicology Chapter of the Tebuthiuron Registration Standard.
The registrant requested Environ Corporation to evaluate the Agency comments and a conference was held on 9/6/88 to discuss the outstanding issues. Environ concluded that although the studies did not meet today's quality criteria, they nevertheless were of sufficient quality to support continued registration of products containing Tebuthiuron (copy attached).

At the Agency's request, both studies were independently reviewed by Dynamac Corporation for scientific merit. Dynamac Corporation concluded that both studies should be classified not more than Supplementary Data (copy attached). The Agency reviewer (D. Ritter) accepted Dynamac's recommendation and concluded that data gaps still exist for the rat and mouse oncogenicity studies (copy attached).

From a meeting between this reviewer, the Acting Division Director and the Acting Herbicide Fungicide Branch Chief, it was decided that additional data were needed to reevaluate the merit of the tebuthiuron 2-year rat and mouse studies. In this action, the requested data were submitted and included:

(1) Inventory of selected tissues from rats given diets containing Tebuthiuron for 2 years
(2) Tabulation of mice with tumors from 15 to 24 months and at study termination from mice given diets containing Tebuthiuron

A. Toxicological Evaluation of Tebuthiuron in Rats for Two Years Studies R-603 and R-613, Lilly Research Lab., 11/1/76

In the 1987 Registration Standard, a number of deficiencies were cited for this study and included:

1. Inadequate survival (less than 25%) at 24 months
2. Numerous instances of "unthrifty" animals
3. Lack of a tissue inventory

1. In this study, the survival rate for the control, low, mid, and high dose was 28%, 16%, 25%, and 31% for males and 25%, 25%, 25%, and 34% for females. Since this study was initiated with a larger number of animals than required (control = 120 per sex, treated groups = 80 per sex instead of 50 per sex/group), the absolute number of animals at final sacrifice for histopathologic evaluation was greater than the minimum number of animals suggested by the Guidelines.

Guidelines : at least	13 males and	13 females
Tebuthiuron control =	33 males	30 females
Tebuthiuron Low Dose =	13 males	20 females
Tebuthiuron Mid Dose =	20 males	20 females
Tebuthiuron High Dose =	25 males	27 females

The absolute number of animals available at final sacrifice would thus be considered as adequate for all groups.

2. Animals in this study had a high incidence of intercurrent disease and were treated with antibiotic during serious outbreak. The incidence of rats with pneumonia approximates 70% in both control and treated groups and undoubtedly is less than desirable based upon today's standards. The question is whether the high incidence of pneumonia would adversely affect the study conclusions.
From the data submitted, body weight, survival, and the tumor incidence apparently were not significantly affected by the health status of the animals.

3. An inventory of histologic sections of selected tissues in control and tebuthiuron treated animals from studies R-603 and R-613 was submitted (11/1/88, authors: Negilski and Todd. EPA Accession No. 408701-01). Based upon this recent submission,

most of the tissues were accounted for histopathologically. A photocopy of the combined inventory of histologic sections is attached (Attachment I).

Recommendation:

Based upon the recent submission of additional data and in consideration of the completion of these studies (R-603 and R-613) prior to the issuance of both 1978 and 1982 FIFRA Guidelines, it is concluded that the submitted data had adequately addressed the oncogenic potential of Tebuthiuron in rats. An oncogenic NOEL could be established at the highest dose level (1600 ppm) with the systemic NOEL at 800 ppm. This study is upgraded to Core Minimum for regulatory purposes.

B. Toxicological Evaluation of Tebuthiuron in Mice for 2 years
Studies M-9153 and M-9163, 11/1/76

A number of study deficiencies were noted in the 1987 Registration Standard and included:

1. Excessive loss of histopathologic data
2. Poor survival
3. Feeding of a foreign compound

1. Histopathology losses due to cannibalism, missing animals and autolysis were higher (22.4%) than today's standards. These losses could have resulted in biasing the conclusions. Most of the losses occurred between 18 and 24 months. To properly assess this question, data showing the numbers of mice with tumors from 15 to 24 months and at study termination were requested. The additional information was submitted by the registrant (authors: Negilski and Todd, 11/1/88). An evaluation of the newly submitted data revealed that the percent of mice with tumors from 15-months to termination (24 months) was neither biologically nor statistically different between control and treated groups for both males and females (Attachment II and III). These data suggest that histopathology losses were equally distributed among groups and did not bias the results. Since this investigation was initiated with larger than normal group sizes (120/sex/control and 80/sex/group instead of 50/sex/group as required), the absolute number of animals available for final histopathologic evaluation was still higher than that required by the Guidelines.

2. At 18 months, the survival rate in this investigation is acceptable even with today's standards.

3. There was a possibility that a foreign compound was administered to the control groups for 3 months. However, based upon body weight and survival data, this possibility is highly unlikely.

Recommendation:

Based upon the newly submitted data and in consideration of the higher than normal group size at study initiation, it is concluded that Tebuthiuron is not an oncogen in mice up to and including a dose level of 1600 ppm. This investigation (studies M-9153 and M-9163) is upgraded to Core Minimum Data.

EPA REG. NO. 10550-1 RIN 0634-93

Page____ is not included in this copy.

Pages 5 through 7 are not included.

The material not included contains the following type of information:

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

TO: Cathy Erumsele, PM # 25
Fungicides/Herbicides Branch
Registration Division TS-767C

THRU: Dr. Quang Bui, Section Head Quang Bui
Rev. Sec. # I/HFASB
Health Effects Division TS-769C (Cover memo attached)

THRU: William L. Burnam, Chief
HFASB
Health Effects Division TS-769C

FROM: D. Ritter, Toxicologist DR 9-29-88
Rev. Sec. # I/HFASB
Health Effects Division TS-769C

Subject: # 1471-101 - Tebuthiuron; response to Data Call-In.

Registrant: Elanco Products Company, Greenfield, IN.

Caswell#: 366AA

TOX Project #: 8-0264

In the Toxicology Chapter of the tebuthiuron Registration Standard we cited a number of deficiencies in certain chronic rodent studies in the toxicity studies submitted by Elanco in the early 70's. The studies are:

Rat Chronic Feeding Study # R-603 and R-613 (MRID 00020714);

Mouse Chronic Feeding Study # M-9153 and M-9163 (MRID 00020717).

Elanco responded by arguing that the studies were valid for the support of continued registration of products containing Tebuthiuron. In response, we reiterated several criticisms in our memos of 3/11/88 and 3/25/88, D. Ritter (copies attached).

The company subsequently had the studies reviewed by the Environ Corporation, an independant outside reviewer, who concluded that, although the studies did not meet today's quality criteria for performing such studies, they nevertheless were of sufficient quality to support continued Registration of products containing tebuthiuron.

We likewise sent the studies for an independant reviewto the Dynamanc Corporation,and they have responded in an evaluation by William L. McLellan, Ph.D. (copy attached). He has concluded for the rat studies (Nos. 603 and 613) that:

1. The major deficiency was the incidence of intercurrent disease, e.g., respiratory infections, requiring treatment of rats with penicillin, and that the incidence of pneumonia was excessive in this strain of rat, and that this could adversely affect the study conclusions.

2. Survival was much lower than seen in todays' studies; even though survival approaches that recommended in the Guidelines, it is not optimal.

3. A tissue inventory was not available so it could not be determined if the absence of an entry in the pathology report indicated whether the tissue was missing or whether it was not examined and found normal.

4. Optic nerves not examined.

5. Less serious deviations from the Guidelines:

   a. Body weights only presented graphically;

   b. Feed consumption data not provided;

   c. Clinical observations were not provided;

   d. Statistical methods inadequately reported.

Overall, Dr. McLellan assessed the rat studies to be no more than CORE Supplementary.

Dr. McLellan concluded that the mouse studies (M9153 and 9163):

1. The number of tissues lost due to autolysis, missing animals or cannabalization was excessive, and therefore an unbiased review of the tumor incidence was not possible.

2. Less serious deviations from the Guidelines:

   a. Minor lesions of aging not reported.

   b. Several important tissues and organs were not routinely examined histopathologically.

   c. Body weight data not supplied.

   d. The possible exposure of control animals to another chemical, although not verified, indicates inadequate GLPs by today's standards.

Dr. McLellan stated that these studies were invalid and should be repeated.

Our Comments:

The Agency accepts the Dynamac verification of its own findings concerning the usefulness of these data, principally because Dynamac is familiar with OPP's data review procedures and Environ is not.

Accordingly, our previous conclusions as to the quality of these studies remains unchanged and data gaps exist for a chronic rat study and a mouse oncogenicity assay for the rangeland herbicide, tebuthiuron.

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EPA: 68D80056
DYNAMAC No. 1-23AB
October 17, 1988

EVALUATION OF RAT AND MOUSE

Oncogenicity Studies on Tebuthiuron

REVIEWED BY:

William L. McLellan, Ph.D.
Dynamac Corporation

Signature: William L. McLellan
Date: Oct. 17, 1988

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: William L. McLellan (for)
Date: October 17, 1988

I. Cecil Felkner, Ph.D.
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 10-17-88

David Ritter
EPA Reviewer, Section I
Toxicology Branch (TS-769C)

Signature: David Ritter
Date: 10-17-88

R. Bruce Jaeger
EPA Section Head, Section I
Toxicology Branch (TS-769C)

Signature: R. Bruce Jaeger
Date: 11-28-88
(cover memo attached)

Rat Studies (Nos. 603 and 613)

BACKGROUND

In the 1987 Registration Standard, a number of deficiencies in the rat chronic studies on terbuthiuron were cited. The major deficiencies were:

1) Survival at 24 months was inadequate (less than 25%) in most groups.

2) There were numerous instances of "unthrifty" animals. The incidence of nephritis, lymphoma, and pneumonia was high in all groups, both dosed and control.

3) A tissue inventory was not present, so that the completeness of histology for each tissue could not be evaluated.

Other lesser deficiencies noted were the lack of food consumption data, no numerical body weight data (only graphic) and no details of statistical analysis were provided.

Environ combined data on survival for both studies and indicated that percent survival at 24 months was less than 25% only in the low-dose group of males (16%). They argued that this did not render the data inadequate since the group size (when both studies were combined) resulted in an absolute number of animals surviving in the low-dose groups that exceeded EPA's recommendation based on a usual group size of 50/sex. Also since the NOEL was set at the mid-dose, the poorer survival at the low dose was not considered to affect the study conclusions.

According to Environ, the incidence of pneumonia in these studies, although high (62%), was normal for Wistar rats. Their review indicated no evidence of unthriftiness in the rats in these studies based on weight gain, survival, and the gross and histologic lesions observed. Environ recognized deviations from guidelines but assessed these to be minor and not to detract from the value of these studies to evaluate chronic toxicity and oncogenicity.

DYNAMAC'S ASSESSMENT

It is this reviewer's assessment that the major deficiency in the rat studies was the incidence of intercurrent disease. It was reported that all animals on study had respiratory infections (acute, subacute, and chronic) and that the colony was treated with penicillin during "serious" outbreaks. The percent of rats with pneumonia at necropsy is shown in Table 1.

Table 1. Percent Increase of Pneumonia on Histologic Examination of Rats Fed Terbuthiuron[a]

	0	400	800	1600
Males				
Study 603	73	61	52.5	52.5
Study 613	70	70	68.5	
Females				
Study 603	75	60	67	42.5
Study 613	65	47.5	55	32.5

[a] There were 60 rats/group in controls and 40 in dose groups.

Environ indicated that this incidence was normal in Wistar rats that were not caesarian derived nor barrier reared. They did not cite historical incidence of pneumonia in Wistar rats but cited an incidence of about 15% in F-344 rats and 26% in Osborne-Mendel rats. Kroes et al. (1981) have compared the histologic profile of untreated conventional Wistar CB rats and a Wistar SPF derived substrain used since 1970. The incidence of pneumonia in conventional non-pathogen free animals (< 18 months old) was 12.7% in males and 17% in females; in SPF Wistar rats (30 months old) the incidence of pneumonia was about 10%. It is our assessment that the incidence of pneumonia for the rats in the terbuthiuron studies was excessive for Wistar derived rats and that this could adversely affect the study conclusions.

The survival of the rats in these studies is much lower than seen with current studies in rats. Even though survival approaches that required by guidelines, this is not optimal. Currrently it is not unusual to see 50-70% survival at 104 weeks and > 20% at 130 weeks.

It is our assessment that the incidence of nephritis and lymphomas in the rats in these studies were not excessively high and did not compromise the histologic evaluation. Lymphoma was present in about 10% of control males and females and the incidence of nephritis (about 16%) was lower than normally found in aging rats. Animals did not appear emaciated.

Kroes, R., Garbis-Berkvens, J. M., deVries, T., Van Nesselrooy, H. J. (1981) Histopathological profile of a Wistar rat stock including a survey of the literature. J. Gerontol. 36: 259-279.

A tissue inventory was not available, so it could not be determined whether the absence of an entry in the pathology report indicated if a tissue were examined and found normal or if it was not examined. There was not an excessive loss of tissues due to autolysis; there were no sections of autolyzed tissues for 6 of 720 animals in the 2 studies.

The optic nerve was not examined; only 2 eyes/sex/group were examined histologically and peripheral nerve was only examined in the case of paralysis. It was indicated in an EPA memo of March 11, 1988, that tissues were not examined unless there were clinical signs or gross lesions. We could not verify this and suggest that a tissue inventory be provided. The protocol stated that minor lesions associated with aging such as atrophy, chronic inflammation, and degeneration were not included in the tables of pathology.

Other less serious derivations from guidelines were:

1) Body weights were only presented graphically and no statistical analyses was provided nor could it be performed in the absence of individual animal data.

2) Food consumption data were not provided.

3) Ophthalmologic examinations were not provided.

4) Clinical observations were not provided.

5) Statistical methods and results were inadequately reported.

6) Clinical pathology data at 6, 12, and 18 months were for 5 animals/sex/group rather than the suggested 10.

In view of the deficiencies, it is our assessment that even with the submission of data to fill the gaps, the study could not be Core classified any higher than Supplementary.

Mouse Studies (Nos. M9153 and M9163)

BACKGROUND

A number of study deficiencies were noted in the 1977 Registration Standard.

1. Necropsy reports for animals that died and those sacrificed at termination were not separated.

2. There were a number of autolyzed or missing tissues/animals and cannibalized animals.

3. The mouse colony was unthrifty.

4. Clinical pathology was performed only at 18 months.

5. Survival at 18 months was inadequate.

6. Control groups may have been exposed to another compound for 3 months.

Environ indicated that EPA was in error concerning survival. The table on survival in the registration Standard was for 24 months and not 18 months. Survival was greater than 60% in all groups at 18 months. Overall survival rate at 24 months was greater than 25%. Environ judged that the losses to histopathology due to cannibalism, missing animals and autolysis (22.4%) was higher than reasonable by today's standards. It was their assessment that the losses were not dose-related and were spread uniformly between groups. Therefore "there was no reason to believe these losses could have resulted in biasing the results". They stated that when results of both studies were combined, the number of animals available for histopathologic examination was larger than currently recommended by guidelines.

Environ disagreed with EPA's evaluation that animals were unthrifty due to a high incidence of nephritis, pneumonia, and malignant lymphoma. Environ pointed out that there was no specific evidence that controls were accidentally fed diets containing 0.36% tricyclazole for 3 months and there were no adverse effects of the type produced by the chemical; however, the possibility could not be ruled out.

DYNAMAC'S ASSESSMENT

This reviewer assesses that survival at 18 months was adequate. If the study had been terminated at 18 months there would possibly have been more tissues for histologic

evaluation. A scan of the pathology tables indicated that about 80% of the animals with tissues lost to autolysis died between 18 and 24 months.

We assess that the incidence of nephritis and malignant lymphoma was not greater than expected in mice in a two year study; the incidence of pneumonia was less than 1%. Therefore we agree with Environ that the mice were not unthrifty.

The number of tissues lost due to total autolysis of animals or cannibalization is assessed to be excessive. This reviewer checked entries of tissue loss and autolysis reported in the pathology tables for individual animals. Our results which differ only slightly from those of Environ are shown in Table 2.

This is a deficiency which cannot be corrected without repetition of the mouse study and it severely compromises any evaluation of histopathologic evaluation.

Table 2. Losses to Histopathology Due to Autolysis, Cannibalism or Missing Tissues of Mice Fed Teruthiuron

	Dietary Level (ppm)			
	0	400	800	1600
Males				
9153	13(22)[a]	8(20)	8(20)	15(30)
9163	16(27)	10(25)	13(33)	9(23)
Females				
9153	15(25)	6(15)	6(15)	9(23)
9163	13(22)	10(25)	5(13)	8(20)

[a] The values in parentheses are percents.

There are several other minor deficiencies in the mouse studies.

1) Trachea, esophagus, cecum, rectum, peripheral nerve, spinal cord, gallbladder and aorta were not routinely examined histopathologically.

2) As in that rat studies, "minor" lesions indicative of aging were not included in the histopathology tables.

3) Body weight data for individual animals was not given, therefore statistical analysis (not provided by sponsor) could not be performed.

4) The fact that the controls were possibly exposed to 3600 ppm Tricyclazole for 3 months cannot be verified or refuted; however, the possibility of this indicates that laboratory practices during the study were far below the present day GLP requirements. Clinical pathology measurements are not required in mouse oncogenicity studies, and absence of measurements at 6 month intervals during the study are not considered a deficiency. From the pathology data, the histologic findings for animals that died/sacrificed moribund and those sacrificed at termination can be separated. In most current studies this data is reported separately and combined.

Our overall assessment is that the excessive loss of tissues due to autolysis; missing animals or cannibalism precludes an unbiased review of the tumor incidence. Therefore the study is invalid and should be repeated.